

# Increased Coronary Atherosclerotic Plaque Vulnerability by Coronary Computed Tomography Angiography in HIV-Infected Men

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**Objective:** Among HIV-infected patients, high rates of MI and sudden cardiac death have been observed. Exploring potential underlying mechanisms, we used multi-detector spiral coronary computed tomography angiography (coronary CTA) to compare atherosclerotic plaque morphology in HIV-infected subjects and non-HIV-infected controls.

**Methods:** Coronary atherosclerotic plaques visualized by CTA in HIV-infected (101) and non-HIV-infected (41) men without clinically apparent heart disease matched on cardiovascular risk factors were analyzed for 3 vulnerability features: low attenuation, positive remodeling, and spotty calcification.

**Results:** 95% of HIV-infected subjects were receiving ART (median duration 7.9 years) and had well-controlled disease (median CD4 473 cells/mm<sup>3</sup>, median HIV RNA <50 copies/ml). Age and traditional cardiovascular risk factors were similar in HIV-infected subjects and controls. Among the HIV-infected (versus control) group, there was a higher prevalence of subjects with at least one: 1) low attenuation plaque (22.8% versus 7.3%,  $p=0.02$ ), 2) positively remodeled plaque (49.5% versus 31.7%,  $p=0.05$ ) and 3) high-risk 3-feature plaque (7.9% versus 0%,  $p=0.02$ ). Moreover, subjects in the HIV-infected (versus control) group demonstrated a higher number of low attenuation plaques ( $p=0.01$ ) and positively remodeled plaques ( $p=0.03$ ) per subject.

**Conclusions:** Our data demonstrate an increased prevalence of vulnerable plaque features among relatively young HIV-infected patients. Differences in coronary atherosclerotic plaque morphology - namely, increased vulnerable plaque among HIV-infected subjects - are here for the first time reported and may contribute to increased rates of MI and sudden cardiac death in this population.

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*AIDS* 2013, **27**:000–000

**Keywords:** atherosclerosis, cardiovascular disease, HIV, myocardial infarction, plaque

## Introduction

While effective antiretroviral therapy (ART) has dramatically extended the lifespan of HIV-infected patients [1] and reduced AIDS-related deaths [2],

cardiovascular disease has emerged as a significant threat to the HIV-infected population [3–5]. Among HIV-infected patients (versus non-HIV-infected controls), the risk of myocardial infarction (MI) is approximately two-fold [6], and the risk of sudden cardiac death is

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Received: 31 October 2012; revised: 23 December 2012; accepted: 9 January 2013.

DOI:10.1097/QAD.0b013e32835eca9b

approximately four-fold [7]. Understanding the mechanisms underlying increased MI and sudden cardiac death risk in HIV-infected patients represents a critical first step toward formulating cardioprotective strategies.

Our research group previously investigated the prevalence of subclinical atherosclerosis by multidetector spiral computed tomography coronary angiography (coronary CTA) in HIV-infected patients without known cardiovascular disease, compared with non-HIV-infected patients matched on traditional cardiovascular risk factors [8]. We showed among the HIV-infected patients a higher prevalence of subclinical atherosclerosis [8]. Moreover, we demonstrated a particularly high prevalence of non-calcified coronary atherosclerotic plaques in the HIV-infected group [9].

MI's and sudden cardiac death, however, do not commonly result from the gradual, progressive expansion of subclinical coronary atherosclerotic plaques. Rather, acute plaque rupture is often culprit, provoking approximately 75% of MI's [10] and 50% of sudden cardiac deaths [11]. Thus, the ability to identify and ultimately predict the presence of coronary atherosclerotic plaque vulnerable to rupture may be important for cardiovascular disease prevention efforts.

Pathologic features characterizing vulnerable plaques include: 1) a necrotic core of lipids and inflammatory cells encased by a thin fibrous cap [11,12], 2) a tendency to remodel eccentrically [12,13], and 3) adherent microcalcifications [14]. The potential of coronary CTA to non-invasively assess these plaque morphologic features is just beginning to be harnessed. Low plaque attenuation in Hounsfield Units (HU) on coronary CTA differentiates lipid-rich plaques from fibrous and/or calcific plaques, as defined by histology [15,16] or intravascular ultrasound (IVUS) [17–20]. Analogously, remodeling indices (ratios of plaque segment diameter to adjacent reference segment diameter) and spotty calcification patterns on coronary CTA correlate with those determined by IVUS [21–24]. Indeed, these three CTA morphology features – low plaque attenuation, positive remodeling (plaque segment diameter > reference segment diameter), and/or the presence of spotty calcification adherent to plaque – have been shown in non-HIV-infected populations to characterize plaques in patients with acute coronary syndrome [25,26] and to prospectively predict plaque rupture [27,28].

In the present study, we extend the scope of our investigation of HIV-infected patients and non-HIV-infected controls through a CTA-based comparative analysis of coronary atherosclerotic plaque morphology. Our aim was to determine whether atherosclerotic plaques in the HIV-infected patients were more vulnerable based on morphologic characteristics. We reasoned that increased vulnerability of atherosclerotic

plaques in HIV-infected patients without clinically apparent cardiovascular disease may contribute to the heightened risk of MI and sudden cardiac death observed in this population. To our knowledge, CTA-based analysis of plaque morphology/vulnerability has not before been undertaken in HIV-infected patients.

## Methods

### Study participants

102 HIV-infected men and 41 non-HIV-infected men age 18–55 were simultaneously recruited from the Boston area. Subjects with known cardiac disease or anginal symptoms were excluded from both groups, as per study design. Additional exclusion criteria included renal dysfunction (creatinine > 1.5 mg/dL, to prevent nephropathy from contrast administered during CTA) and contraindication to iodinated contrast medium/beta blocker/nitroglycerin. HIV-infected subjects on ART were required to have been on a stable regimen for > 3 months. An effort was made to match subjects in both groups on traditional cardiovascular risk factors including age, blood pressure, lipids, and smoking. Institutional Review Boards from the Massachusetts General Hospital (Partners Healthcare) and Massachusetts Institute of Technology approved the study, and informed consent was obtained from all subjects. Data on the prevalence of subclinical atherosclerosis in association with inflammatory biomarkers were previously published [8,9], but data on atherosclerotic plaque vulnerability in this cohort have never before been reported.

### Assessment of historical data, body composition, and metabolic and immunologic parameters

Data on HIV diagnosis and prior ART, nadir CD4, and Hepatitis C infection status, as well as past medical history, medications, behaviors (e.g. smoking), and family history, were elicited. During a fasting morning visit, all subjects underwent weight and anthropometric measurements and fasting blood draws. Body mass index (BMI) was calculated. Waist circumference was measured at the iliac crest. Standard techniques were used to determine lipid and glucose levels. Flow cytometry was used to assess CD4+ T cell counts while ultrasensitive reverse-transcription polymerase chain reaction (PCR) was used to assess HIV viral load (Roche Amplicor Monitor; lower limit of detection = 50 copies/ml). Enzyme-linked immunosorbent assay (ELISA) was used to evaluate levels of IL-6 (R&D), MCP-1 (R&D), sCD163 (Trillium Diagnostics), and sCD14 (R&D). High sensitivity CRP was assessed by the Cobas Integra C-Reactive Protein (Latex) Test. The endpoint LAL assay (Associates of Cape Cod) was employed to determine levels of lipopolysaccharide (LPS). An immuno-turbidometric method was performed for quantitative assessment of D-dimer levels.

Visceral adipose tissue area was assessed by single-slice abdominal CT scanning at L4-L4 [8].

### Multidetector row computed tomography coronary angiography (coronary CTA)

In all subjects, coronary CTAs were obtained for research purposes and not for clinical indications. A 64-slice CT scanner (Sensation 64; Siemens Medical Solutions, Forchheim, Germany) was used to obtain CT images as per our previously published protocols [8,9] (See Supplement 1 on Coronary CTA Imaging Methodology, <http://links.lww.com/QAD/A305>). Coronary CTA images from one HIV-infected subject were deemed to be of inadequate quality such that coronary atherosclerotic plaque morphology data from 142 subjects were analyzed.

### Assessment of plaque vulnerability features by coronary CTA

All coronary arterial segments previously identified as having any plaque were re-evaluated by trained experts (S.A., B.W.), who were blinded to subjects' HIV status. Cross-sectional and multiplanar reconstructed images were acquired and assessed for 18 coronary artery segments: left main coronary artery, proximal, mid, and distal left anterior descending artery segments, 1<sup>st</sup>-3<sup>rd</sup> diagonal branches, ramus intermedius, proximal, mid, and distal left circumflex coronary artery segments, 1<sup>st</sup> and 2<sup>nd</sup> obtuse marginal branches, posterior left ventricular branch, proximal, mid, and distal right coronary artery segments, and posterior descending artery. Proximal arterial segments were defined as left main coronary artery, proximal left anterior descending artery segment, proximal left circumflex coronary artery segment, and proximal right coronary artery segment. Low attenuation plaque was defined as plaque with a mean minimal attenuation < 40 Hounsfield Units [26]. For determination of low attenuation plaque, 5 regions of interest

(area = 1 mm<sup>2</sup>) were placed on each plaque and the smallest average value within the regions of interest was recorded to represent the mean minimal attenuation. Positive remodeling was defined as [plaque segment diameter/ reference segment diameter] > 1.05 [26]. Spotty calcification was defined as calcification < 3 mm in size on multiplanar reconstructed images and occupying just 1 side on cross-sectional images [27]. The lower limit of detection for calcification size was approximately 0.5 mm. Cut-points for plaque vulnerability features were drawn from literature among non-HIV-infected subjects relating vulnerability as defined by the specific cut-points to cardiovascular endpoints [26,27]. Representative examples of the appearance of coronary atherosclerotic plaques with low attenuation, positive remodeling, or spotty calcification are shown (Fig. 1).

### Statistical analysis

#### Baseline demographics

Normally distributed data are presented as mean ± standard deviation; non-normally distributed data as median (interquartile range). Between-group comparisons were performed using the Student's t test for normally distributed variables, and using the Wilcoxon rank sum test for non-normally distributed variables. Dichotomous parameters were compared between groups using chi square test likelihood ratios.

#### Plaque vulnerability features

In our primary analyses, comparisons of plaque features were made on a per subject basis between groups. Comparison of the prevalence of HIV-infected versus non-HIV-infected subjects with at least one type of vulnerable plaque and with at least one 3-feature plaque was made using the chi square test and determination of the likelihood ratio. Comparison of the numbers of total vulnerable plaque types per subject and proximal arterial segment vulnerable plaque types per subject between the

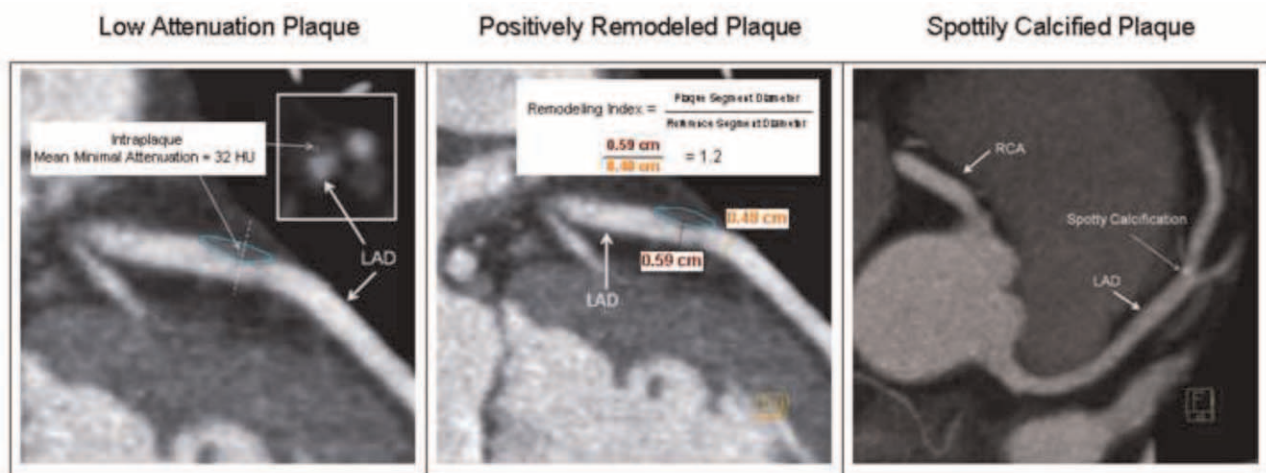


Fig. 1. Characteristic examples of plaques with vulnerability features including low attenuation (panel a), positive remodeling (panel b), and spotty calcification (panel c).

HIV-infected and non-HIV-infected groups was made using the Wilcoxon test. The degree to which particular vulnerability features clustered with one another was determined by chi square test likelihood ratio. Univariate regression analysis was performed to determine demographic, metabolic, and immunologic parameters related to the number of low attenuation plaques per subject separately within the HIV-infected and non-HIV-infected groups. In these analyses, non-normally distributed parameters were related using Spearman's rho, or were log-transformed and related via the Pearson correlation coefficient. Multivariate regression modeling for the number of low attenuation plaques per subject was performed among the entire cohort controlling for HIV status, and again among HIV-infected subjects and non-HIV-infected subjects separately. Independent variables entered into the model included traditional cardiovascular risk factors, as well as HIV-specific and immunologic parameters for the analyses within the HIV-infected group. In a Supplemental Analysis, we compared

the number of affected vulnerable plaque segments between groups (HIV-infected versus non-HIV-infected). In this segment-based analysis, as opposed to the primary subject-based analyses above, we used conditional logistic regression analysis to account for the potential interdependence of observations in the eighteen arterial segments within individual subjects. All statistical analyses were performed using SAS (JMP 9.0) (SAS Institute). Two-tailed probability values were assessed, with  $p < 0.05$  considered statistically significant.

## Results

### Characteristics of participants

102 HIV-infected men and 41 non-HIV-infected men underwent MDCT coronary angiography. Baseline characteristics of the study participants are described in Table 1. The average duration of HIV disease was

**Table 1. Baseline Demographics.**

Characteristic	Non-HIV-infected controls (n = 41)	HIV-infected subjects (n = 102)	p-value
<b>Demographics and Cardiovascular Risk Factors</b>			
Age, years	45.0 (41.5, 50.5)	48.0 (41.8, 52.0)	0.18
Race			0.19
White	61	63	
Black	17	22	
Hispanic	7	12	
Asian	7	1	
Native American	5	3	
Family history of premature CHD by NCEP (%)	13	22	0.24
Current smoker (%)	29	41	0.20
Diabetes mellitus (%)	2	7	0.26
Use of antihypertensive medications (%)	8	29	0.003
Use of lipid-lowering medications (%)	13	27	0.05
Hepatitis C infection (%) (self-reported)	2	25	0.0003
BMI, kg/m <sup>2</sup>	26.3 (23.5, 29.6)	25.7 (23.7, 29.1)	0.75
Waist circumference, cm	93.9 (84.0, 104.6)	97.6 (86.7, 108.4)	0.33
Visceral adipose tissue, cm <sup>2</sup>	144.8 (60.0, 186.6)	133.6 (77.4, 241.1)	0.55
Systolic blood pressure, mm Hg	117 (110, 124)	120 (112, 127)	0.22
Diastolic blood pressure, mm Hg	76 ± 9	77 ± 9	0.60
Total cholesterol level, mg/dL	177 (154, 201)	173 (155, 201)	0.94
HDL cholesterol level, mg/dL	46 (41, 52)	45 (38, 55)	0.75
LDL cholesterol level, mg/dL	110 ± 33	101 ± 31	0.15
Triglyceride level, mg/dL	80 (64, 123)	109 (81, 182)	0.001
<b>HIV disease-related parameters</b>			
Duration of HIV infection, years	N/A	13.8 ± 6.5	N/A
Currently receiving ART (%)	N/A	95	N/A
Duration of antiretroviral therapy, years	N/A	7.9 (3.1, 11.0)	N/A
Current PI treatment (%)	N/A	52	N/A
Current NRTI treatment (%)	N/A	92	N/A
Current NNRTI treatment (%)	N/A	47	N/A
Current CD4+ count, cells/mm <sup>3</sup>	N/A	473 (303, 748)	N/A
Nadir CD4 count, cells/mm <sup>3</sup> (self-reported)	N/A	175 (57, 278)	N/A
HIV RNA level (viral load, VL), copies/mL	N/A	<50 (<50 to <50)	N/A
Undetectable VL (<50 copies/mL) (%)	N/A	81	N/A
<b>Immune Activation Markers</b>			
C-reactive protein, mg/L	1.3 (0.7, 3.3)	1.6 (0.7, 3.8)	0.49
Hs interleukin-6, pg/mL	0.6 (0.5, 1.0)	0.9 (0.7, 1.5)	0.01
D-dimer, ng/mL	<220 (<220, 333)	<220 (<220, 322)	0.93
LPS, ng/mL	0.07 (0.06, 0.1)	0.1 (0.07, 0.1)	0.0004
MCP-1, pg/mL	235 (190, 299)	275 (179, 363)	0.13
sCD163, ng/mL	765 (572, 1054)	1063 (695, 1577)	0.0007
sCD14, ng/mL	211 (121, 374)	305 (157, 440)	0.08

13.8 years. 95% of these subjects were receiving ART therapy, and the median duration of therapy was 7.9 years. The HIV-infected subjects were under good immunologic control, with a median CD4+ count of 473 cells/mm<sup>3</sup>. HIV VL was undetectable (<50 copies/mL) in 81%. Age, race, family history of premature CAD, smoking rates, prevalence of diabetes, body mass index (BMI), waist circumference, visceral adipose tissue, blood pressure, and levels of total, HDL, and LDL cholesterol were not significantly different between the groups. The HIV-infected group had more prevalent use of anti-hypertensive ( $p = 0.003$ ) and lipid-lowering medications ( $p = 0.05$ ). There was a higher percentage of subjects with self-reported Hepatitis C infection in the HIV-infected group (25% versus 2%,  $p = 0.0003$ ).

### Analysis of coronary CTA data

#### *Prevalence of subclinical coronary atherosclerosis*

As previously reported, a significant difference in the prevalence of subclinical coronary atherosclerosis by coronary CTA was noted between the two groups [9]: With data from 142 subjects analyzed, 59.4% ( $n = 60$ ) in the HIV-infected group were noted to have plaque in the coronary arteries versus 39.0% ( $n = 16$ ) among controls ( $p = 0.03$ ).

#### *Prevalence of plaque vulnerability features*

A higher prevalence of subjects with at least one low attenuation plaque (22.8% versus 7.3%,  $p = 0.02$ ) and with at least one positively remodeled plaque (49.5% versus 31.7%,  $p = 0.05$ ) was observed in the HIV-infected group. There was no difference between groups in prevalence of subjects with at least one spottily calcified plaque (32.7% versus 29.3%,  $p = 0.69$ ) (Fig. 2). A higher prevalence of subjects with at least one high-risk - 3-feature positive - plaque was demonstrated in the HIV-infected group (7.9% versus 0%,  $p = 0.02$ ) (Fig. 2). Of note, atherosclerotic plaque segments with low attenuation were more likely than those without low attenuation to feature positive remodeling among segments with plaque (83% vs. 52%,  $p < 0.0001$ ). In contrast, there was no apparent interrelationship between those segments with low attenuation and spotty calcification ( $p = 0.44$ ) or between those segments with positive remodeling and spotty calcification ( $p = 0.52$ ) among segments with plaque. Between group comparison (HIV versus non) of the number of plaque segments with vulnerability features among all segments is included as a Supplemental Analysis (Supplement 2, <http://links.lww.com/QAD/A305>), and provides similar results to the per subject analysis.

#### *Frequency of plaque vulnerability features*

Compared with subjects in the control group, subjects in the HIV-infected group demonstrated a higher number of low attenuation plaques per subject ( $p = 0.01$ ). The percentage distribution of the number of low attenuation plaques per subject by group is shown in Supplemental

Figure 1a, <http://links.lww.com/QAD/A305>. Subjects in the HIV-infected group also demonstrated a higher number of positively remodeled plaques per subject ( $p = 0.03$ ). The percentage distribution of the number of positively remodeled plaques per subject by group is shown in Supplemental Figure 1b, <http://links.lww.com/QAD/A305>. Moreover, subjects in the HIV-infected group demonstrated a higher number of low attenuation plaques per subject ( $p = 0.02$ ) and positively remodeled plaques per subject ( $p = 0.05$ ) in the proximal arterial segments. There was no statistically significant difference in the number of spottily calcified plaques per subject between groups ( $p = 0.12$ ). The overall number of low attenuation plaques per subject remained significantly different between HIV-infected and non-HIV-infected subjects ( $p = 0.03$ ), controlling for traditional risk factors including age, family history of premature CHD, smoking, diabetes, systolic blood pressure, and LDL cholesterol, and controlling for Hepatitis C status (Supplemental Table 1, <http://links.lww.com/QAD/A305>).

### Assessment of demographic, metabolic, and immunologic parameters in relation to plaque vulnerability (number of low attenuation plaques per subject)

#### *Univariate regression analyses*

Demographic, metabolic, and immunologic parameters were assessed via univariate regression in relation to plaque vulnerability - as reflected in number of low attenuation plaques per subject among non-HIV-infected and HIV-infected groups separately. These parameters included age, pack-years cigarette smoking, body mass index, systolic blood pressure, fasting glucose, HDL cholesterol, LDL cholesterol, triglycerides, C-reactive protein, high-sensitivity interleukin-6, D-dimer, LPS, MCP-1, sCD163, and sCD14. Among non-HIV-infected subjects, body mass index ( $\rho = 0.36$ ,  $p = 0.02$ ) and systolic blood pressure ( $\rho = 0.34$ ,  $p = 0.03$ ) were significantly related to the number of low attenuation plaques per subject (Table 2). Among the HIV-infected subjects, the same parameters were tested, as well as HIV-specific parameters including duration of HIV infection, duration of ART, CD4 count, nadir CD4 count, and viral load. In this group, age ( $\rho = 0.28$ ,  $p = 0.004$ ), systolic blood pressure ( $\rho = 0.23$ ,  $p = 0.02$ ), LDL cholesterol ( $r = 0.22$ ,  $p = 0.03$ ), triglycerides ( $\rho = 0.21$ ,  $p = 0.04$ ), log sCD163 ( $r = 0.22$ ,  $p = 0.03$ ), duration of HIV infection ( $r = 0.22$ ,  $p = 0.02$ ), and duration of ART ( $\rho = 0.34$ ,  $p = 0.006$ ) were significantly related to the number of low attenuation plaques per subject (Table 3).

#### *Multivariate regression modeling*

Multivariate regression models were constructed to assess relationships between demographic, metabolic, and immunologic parameters and plaque vulnerability - as reflected in number of low attenuation plaques per subject

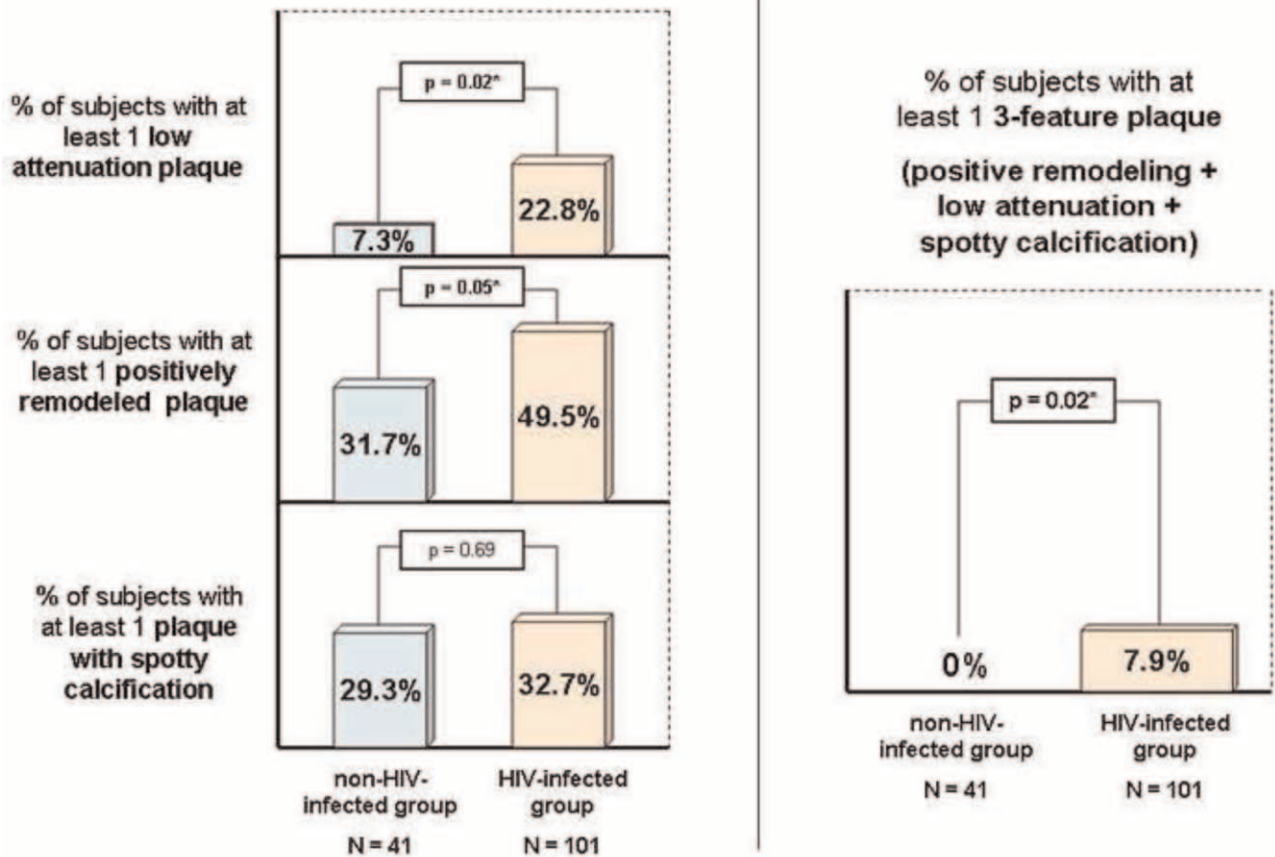


Fig. 2. Prevalence of HIV-infected and non-HIV-infected subjects with at least one coronary atherosclerotic plaque positive for select vulnerability features.

separately among non-HIV-infected and HIV-infected subjects.

For non-HIV-infected subjects, independent variables entered into the model included those variables significant on univariate analysis, as well as traditional

Table 2. Association between Demographic, Metabolic, and Immunologic Factors versus Number of Low Attenuation Plaques per Subject among Non-HIV-infected Subjects.

Variable	Correlation Coefficient	p value
Age	rho = 0.29	0.07
Pack years smoking	rho = -0.15	0.37
Body mass index	rho = 0.36	0.02*
Systolic blood pressure	rho = 0.34	0.03*
Fasting glucose	rho = 0.09	0.56
HDL cholesterol	rho = -0.04	0.80
LDL cholesterol	r = 0.19	0.23
Triglycerides	rho = 0.24	0.13
C-reactive protein	rho = -0.04	0.78
Hs interleukin-6	rho = -0.15	0.42
D-dimer	rho = 0.26	0.10
LPS	rho = -0.16	0.30
MCP-1	rho = -0.10	0.56
log sCD163	r = 0.19	0.23
sCD14	rho = -0.12	0.46

cardiovascular risk factors. None of the variables remained significantly related to the number of low attenuation plaques per subject in this group. For HIV-infected subjects, independent variables entered into the model included those variables significant on univariate analysis, traditional cardiovascular risk factors, as well as demographic and immune parameters related to HIV infection - Hepatitis C status, duration of ART, nadir CD4, viral load, and sCD163. sCD163 was tested for entry into the model because it was the only immune activation marker found to be associated with number of low attenuation plaques per subject on univariate analysis. In this multivariate model among HIV-infected subjects ( $R^2 = 0.49$ ,  $p = 0.03$ ), only sCD163 remained significantly related to number of low attenuation plaques per subject when controlling for traditional cardiovascular risk factors as well as demographic and immune parameters related to HIV infection ( $\beta$ -estimate 0.001,  $p = 0.009$ ) (Table 4).

## Discussion

The present investigation builds on our previous findings of increased non-calcified coronary plaque burden among

**Table 3. Association between Demographic, Metabolic, and Immunologic Factors versus Number of Low Attenuation Plaques per Subject among HIV-Infected Subjects.**

Variable	Correlation Coefficient	p value
Age	rho=0.28	0.004*
Pack years smoking	rho=-0.007	0.94
Body mass index	rho=0.04	0.71
Systolic blood pressure	rho=0.23	0.02*
Fasting glucose	rho=0.03	0.74
HDL cholesterol	rho=-0.05	0.60
LDL cholesterol	r=0.22	0.03*
Triglycerides	rho=0.21	0.04*
C-reactive protein	rho=0.04	0.71
Hs interleukin-6	rho=0.09	0.39
D-dimer	rho=-0.12	0.24
LPS	rho=0.02	0.85
MCP-1	rho=0.16	0.12
log sCD163	r=0.22	0.03*
sCD14	rho=0.16	0.11
Duration HIV	r=0.22	0.02*
Duration of ART	rho=0.34	0.006*
CD4 count	rho=-0.03	0.80
nadir CD4 count	rho=-0.11	0.32
log viral load	r=0.05	0.67

HIV-infected patients without clinically apparent heart disease (versus non-HIV-infected controls with similar traditional cardiovascular risk factors). Specifically, we now demonstrate that relatively young HIV-infected patients with well-controlled disease have not only a higher burden of plaque but also a higher burden of vulnerable plaque relative to controls with similar traditional cardiovascular risk factors. Specifically, among HIV-infected group (versus controls), a higher prevalence of patients with at least one: 1) low attenuation plaque, 2) positively remodeled plaque, and 3) high-risk 3-feature positive plaque was seen.

The clinical significance of the increased plaque vulnerability has been investigated in symptomatic non-HIV-infected patients: Kitagawa et al. showed that coronary plaques in acute coronary syndrome (ACS) patients were more frequently characterized by low attenuation, positive remodeling, and spotty calcification than plaques in non-ACS patients undergoing coronary

**Table 4. Multivariate Model for Number of Low Attenuation Plaques Per Subject Among HIV-Infected Subjects Only.**

Parameter	Estimate	Standard Error	p value
Age	-0.004	0.03	0.90
Family history of premature CHD	-0.21	0.24	0.39
Current smoking	0.27	0.21	0.22
Current diabetes	-0.31	0.67	0.65
Systolic blood pressure	0.02	0.02	0.20
LDL cholesterol	0.003	0.007	0.64
Hepatitis C status	0.76	0.38	0.06
Duration of ART	0.07	0.04	0.11
Nadir CD4 count	-0.0004	0.001	0.72
Viral load	-3.9e-5	2.5e-5	0.14
sCD163	0.001	0.0004	0.009*

$R^2 = 0.49$   $p = 0.03$ .

CTA on clinical grounds. Moreover, the frequency of non-calcified coronary plaques characterized by all 3 features was two-fold higher in ACS versus non-ACS patients (42% versus 22%,  $p < 0.01$ ) [26]. Similarly, Motoyama et al. showed that coronary plaques in ACS patients were more frequently characterized by low attenuation, positive remodeling, and spotty calcifications than plaques in patients with stable angina. Among the ACS patients studied, the presence of all 3 features had high positive predictive value to identify the “culprit,” or recently ruptured, lesion [25]. In a landmark follow-up study, Motoyama et al. showed that among patients with known or suspected coronary disease who had undergone coronary CTA, those patients with 2-feature positive plaques (low attenuation and positive remodeling) went on to develop ACS at a rate of 22.2% over 2 years. This ACS rate dramatically exceeded that observed in patients with 1-feature positive plaques (3.7%) and plaques with no vulnerability features (0.5%). Not surprisingly, both low attenuation plaque and/or positively remodeled plaque independently, prospectively predicted ACS in this cohort [27]. Moreover, in a prospective study by Nakanishi et al. assessing the ability of coronary CTA-based plaque morphology features to predict cardiovascular events among patients with hypertension, the feature of low attenuation was found to predict ACS more accurately than traditional cardiovascular risk factors [28]. Further studies are needed to determine the potential of CTA-based plaque vulnerability features to predict plaque rupture/ACS among HIV-infected patients without clinically apparent cardiovascular disease.

Other studies among the general population have shown that in the absence of a known immune activation state (such as HIV), traditional cardiovascular risk factors relate to CTA-based plaque vulnerability features on multivariate analysis. Specifically, among patients with intermediate or high Framingham Risk Scores who are either asymptomatic or experience atypical chest pain, factors of male sex, diabetes, and current smoking were associated with vulnerable plaque (as defined by low attenuation and positive remodeling) [29]. Moreover, among asymptomatic patients with type II diabetes, current smoking was once again associated with vulnerable plaque, defined similarly [30]. In the present analysis, traditional cardiovascular risk factors were found to relate to plaque morphology among HIV-infected and non-HIV-infected patients in univariate regression analysis. However, on multivariate regression modeling for number of low attenuation plaques per subject in a pooled analysis of both groups, HIV status remained significantly related to plaque morphology controlling for traditional cardiovascular risk factors while traditional cardiovascular risk factors were no longer significant. This finding suggests that differences in traditional cardiovascular risk factors do not account for differences in vulnerable plaque between HIV-infected and non-HIV-infected groups.

We have previously shown marked arterial inflammation on cardiac FDG-PET scanning in association with high levels of the monocyte activation marker sCD163 [31] among relatively young, well-treated HIV-infected patients without clinically apparent cardiovascular disease and with low Framingham Risk Score. These findings support an emerging hypothesis that systemic immune activation - in addition to a preponderance of traditional risk factors - may contribute to increased MI and sudden cardiac death rates among HIV-infected patients [32,33]. Intriguingly, in the present study, we note that sCD163 levels were significantly related to the number of low attenuation plaques per patient among HIV-infected patients (in contrast to all other immune activation markers which were tested and found not to be related). Moreover, in multivariate regression modeling among HIV-infected patients in the current study, sCD163 was found to be related to the number of low attenuation plaques per patient even when controlling for traditional cardiovascular risk factors (age, family history of premature CHD, smoking, diabetes, systolic blood pressure, LDL cholesterol) and HIV-specific factors (Hepatitis C status, duration of ART, nadir CD4 count, and viral load). These data extend our prior data and relate sCD163 to vulnerable plaque morphology for the first time among HIV-infected patients. This observation has biologic plausibility given that activated monocytes participate in internalization of oxidized LDL particles in the atheroma core and in degradation (via secretion of matrix metalloproteinases) of the atheroma's protective fibrous cap [34-36]. The correlation of systemic monocyte activation markers with both arterial inflammation (on cardiac PET) shown in a prior study from our group [31] and now with plaque vulnerability features on coronary CTA in the current study suggest the need for a direct comparison of arterial inflammation by PET and plaque morphologic features by CTA in future studies.

To date, the only prospective studies relating CTA-based atherosclerotic plaque morphologic features to cardiac events have been conducted among patients in the general population with known or suspected heart disease [27] or with specific traditional cardiovascular risk factors such as hypertension [28]. At this time, prospective studies relating these features to cardiac events among HIV-infected men and women with and without known heart disease are needed. Moreover, it will be important to identify circulating immune markers predictive of atherosclerotic plaque vulnerability among even those HIV-infected patients with low traditional cardiovascular risk. In this regard, sCD163 is a promising marker that requires further study.

Study limitations include the cross-sectional design, the focus on male patients without renal dysfunction, and the relatively small sample size. A marginally higher percentage of HIV-infected patients were receiving

lipid-lowering therapy (including statins), but among non-HIV-infected patients, statins have been shown to increase plaque attenuation and decrease plaque remodeling [37], such that any excess lipid-lowering medication use by HIV-infected patients would be expected to minimize plaque vulnerability differences between the groups. Strengths of the study include the novel application of coronary CTA to assess atherosclerotic plaque morphology among HIV-infected patients and the ability to compare plaque morphology between HIV-infected patients and non-HIV-infected patients with similar traditional cardiovascular risk factors.

In summary, our study represents the first analysis of CTA-based coronary atherosclerotic plaque morphology among HIV-infected patients compared to non-HIV-infected controls. We find an increased prevalence of plaque vulnerability - including low attenuation plaque, positively remodeled plaque, and high-risk 3-feature positive plaque - among HIV-infected patients versus controls with similar traditional cardiovascular risk factors. We also find a higher number of low attenuation plaques and positively remodeled plaques per patient among the HIV-infected group. In light of the potential of vulnerable plaque to rupture, resulting in clinically significant cardiac events, our results highlight a potential mechanism for heightened rates of MI's and sudden cardiac death [7] in this population. Additionally, we find a relationship between the immune activation marker sCD163 and the number of low attenuation plaques per patient, and show that this relationship holds even when controlling for traditional cardiovascular risk factors and HIV-specific parameters.

## Acknowledgements

### Conflicts of interest

None declared.

Clinical Trial Registration Number: NCT 00455793.

Author contributions: Study design (M.V.Z., S.A., J.L., and S.K.G.), data collection (M.V.Z., S.A., J.L., B.W., D.H., E.M., and S.K.G.), data interpretation (M.V.Z., S.A., E.M., and S.K.G.), drafting of manuscript (M.V.Z. and S.K.G.), critical revision of manuscript (M.V.Z., S.A., J.L., B.W., D.H., E.M., and S.K.G.). All authors have read and approved the text submitted.

Grant Support: This work was conducted with the support of a Medical Research Investigator Training (MeRIT) award from Harvard Catalyst | The Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award 8KL2TR000168-05. The content is



solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic health care centers, or the National Institutes of Health. This work was also conducted with research funding from Bristol Myers Squibb to Dr. Grinspoon and with the support of National Institutes of Health Grants K23 HL092792 to Dr. Lo, K24 DK064545 and RO1HL095123 to Dr. Grinspoon, and M01-RR-01066 and 1 UL1 RR025758-01, Harvard Clinical and Translational Science Center, from the National Center for Research Resources. Finally, this work was supported in part by the NIH grant P30DK040561 to the Nutrition Obesity Research Center at Harvard.

Disclosures: Dr. Grinspoon received research funding for this investigator-initiated research project through Bristol Myers Squibb, Inc. Funding sources had no role in the design of the study, data analysis, or the writing of the manuscript. There are no other author disclosures relevant to this manuscript.

Author contributions: M.V.Z., S.A., and S.K.G. contributed to study design, data collection, data interpretation, drafting of manuscript, and critical revision of manuscript.

J.L. contributed to study design, data collection, and critical revision of manuscript.

E.M. contributed to data collection, data interpretation, and critical revision of manuscript.

B.W. and D.H., contributed to data collection and critical revision of manuscript.

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